II. REMARKS

Preliminary Remarks:

The official action has not included claim 48 in the stated rejections, but has not indicated that claim 48 is allowable. Clarification of the status of claim 48 is respectfully requested.

Non-elected claims 26 to 38 are canceled without prejudice. New claims 49 to 56 are directed to the method for treating or preventing tissue damage in a subject having an inflammatory and/or tissue damaging condition according to claims 1, 10, and 18, wherein the inflammatory and/or tissue damaging condition comprises a stroke, as described on page 5, line 3. The new claims are within the restriction of the claims to inflammatory and/or tissue damaging condition comprising atherosclerosis, as stroke and ischemic injury are known to be physiological results of the occlusion of blood vessels of the brain by atherosclerotic plaques and by the clots that form at sites of atherosclerotic plaques. Foe example, see the description of stroke secondary to atherosclerosis in the "Medline Plus" medical encyclopedia, which is available in the web site of the U.S. National Library of Medicine (copy attached).

Patentability Remarks:

35 U.S.C. §112, First Paragraph

Claims 1-9, 16-19, 39-41, and 44 to 46 were rejected under 35 U.S.C. §112, First Paragraph, on the basis that the specification "does not reasonably provide enablement" for compounds defined as being capable of inhibiting the binding of C-reactive protein to an autologous or extrinsic ligand thereof. The Examiner does, however, believe that the compound as defined in claim 10 would be enabled. The Examiner appears to base this objection on a contention that (*i*) only a limited number of inhibitory compounds are set forth, (*ii*) that the compounds are defined by their function and no information as to the structure-activity relationship is provided, and (*iii*) that a person of ordinary skill would have been required to perform undue experimentation to identify such compounds.

We respectfully disagree with the Examiner for at least the following reasons:

It is set out in section 2164 of the US Manual of Patent Examining Procedure (MPEP) that "[d]etailed procedures for making and using the invention may not be necessary if the description of the invention itself is sufficient to permit those skilled in the art so they can use the invention". According to section 2164.02 on working examples, an applicant need not have actually reduced the invention to practice prior to filing. It is indicated that the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. It is further indicated that an applicant need not describe all actual embodiments. In MPEP § 2164.01(b) ("Working Examples and a Claimed Genus"), it is indicated that, for a claim to genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art would expect the claimed genus could be used in that manner without undue experimentation. It appears necessary to consider the level of skill of one skilled in the art, the state of the art and the information in the specification.

Turning now to the present specification, it is submitted that sufficient information is provided by the present application in relation to C-reactive protein (CRP) and its inhibitors to enable persons skilled in the art to make and use the claimed invention without undue experimentation. Claim 1 requires the inhibitory compound of the claim to be capable of inhibiting the binding of CRP to a ligand thereof. The ligand may be autologous or extrinsic. At the time the present invention was made, the CRP molecule was very well characterised. The central paragraph on page 12 of the specification indicates that the CRP molecule was characterised to atomic resolution. The skilled addressee would therefore know the three-dimensional structure of the molecule. It is indicated in line 6 of this paragraph that the CRP molecule has a specific calcium-dependent binding site through which it binds to its ligands. The major class of compounds that can be identified using the invention comprises substances that are bound by this calcium-dependent ligand binding site of CRP so as to interfere with binding of CRP to its ligands. The specification indicates here that the binding site has been characterised by X-ray crystallography at atomic resolution and prior art references are given. The skilled addressee would therefore have very clear knowledge of the three dimensional structure of the binding sites on CRP to which inhibitors would bind.

This paragraph also mentions that knowledge of the three-dimensional structure of CRP provides for molecular design of model compounds useful in the present invention. WO95/05394 is cited as one method by which inhibitory compounds may be designed. Other methods using structure-based drug design are indicated also to be useful. In the last sentence of this paragraph it is mentioned that the hydrophobic cleft adjacent to the site in which phosphocholine, the highest affinity natural ligand for CRP is bound, is also a suitable target for drug design. It follows that the compound of general formula 1 set out on page 13 and claimed in claim 10 is just one example of a broader class of compounds capable of inhibiting CRP binding to its ligands.

According to page 8, the invention is also concerned with the method for selecting a pharmaceutical compound by testing for CRP ligand binding in the presence of a test compound. This is a routine assay methodology, the goal of which is to find compounds inhibitory to CRP ligand binding. Details of the methodology are set out on pages 9 to 11. Page 9 sets out the basic reaction conditions and page 10 sets out a typical binding assay using a solid phase. Assays of this nature would be routine to those of skill in this particular art.

Specific examples of methods for showing binding of CRP to its ligands are set out on pages 14 to 20. On page 15 various ligands are set out which may be used in assays for inhibitory compounds.

Accordingly, we submit that sufficient working examples are provided in the present specification to enable the skilled addressee to provide a variety of compounds capable of inhibiting CRP binding to ligands. The level of one of ordinary skill is high in this technical field. The skilled addressee would also have available a significant amount of information concerning the structure of CRP and the testing methodologies to provide inhibitory compounds claimed. This information is provided by the state of the art, as acknowledged in the specification. This information is also provided by the extensive teaching of the specification.

The Examiner has suggested that no information is provided as to the structure-activity relationship of the inhibitors of the invention. This is incorrect. For example, the simplest form of inhibitor would be one capable of blocking the site of interaction between

CRP and its target ligand by binding at the calcium-dependent binding site. As mentioned above, the structure of this binding site has been characterised to atomic resolution by X-ray crystallography. Here, the structure-activity relationship would be absolutely clear to the skilled addressee. The compounds of general formula I represent just one class of compounds capable of being bound at the ligand binding site of CRP. Other compounds described in the present specification would also be capable of being bound at this site. Such compounds include the ligands set out on page 15 of the specification. These ligands, and synthetic analogues thereof, are capable to varying degrees of inhibiting the binding of CRP to other autologous or extrinsic ligands in exactly the same way as the compounds of general formula 1. The skilled addressee would therefore have a range of potentially useful inhibitory compounds for testing and ultimately using in accordance with the invention.

The Examiner has also alleged that the persons of skill in the art pertaining to the claimed invention would have been required to perform undue experimentation to identify compounds within the claimed scope. As set out in the MPEP under 2164.01, the fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. Moreover, the test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. We submit that, in the present technical field the level and complexity of experimentation set out in the present specification would not have been undue. The direction and guidance found in the specification is fully sufficient to enable one of skill in the art to obtain compounds that inhibit the binding of CRP to its ligands, as required in 2164.01(a). As set out above, assay procedures are to be found set out in detail in the specification. As has already been submitted, a high level of skill in the art would be expected for one of ordinary skill. Moreover, all of the methods needed to obtain or make inhibitory compounds were well known at the time of the invention. Importantly, the present specification not only describes compounds of general formula I but also describes assay methodology normal in the art to extend the teaching of general formula I further. Starting from these compounds, analogues thereof, or other compounds designed rationally using the methodology described in the present specification, a skilled addressee would not be required to perform experimentation beyond what would normally be expected in this technical field.

35 U.S.C. §103(a)

Claims 1-25 and 42-47 (and presumably claim 48) were rejected under 35 U.S.C. §103(a) as being obvious in view of Bhakdi et al. and Kitao et al., further in view of U.S. Patent No. 5,064,817 of Yedgar et al. ("the Yedgar patent"), further in view of U.S. Patent No. 4,640,913 of Wissner et al. ("the Wissner patent"). The applicants respectfully traverse the rejection.

First of all, it is crucial to appreciate what the common general knowledge of the skilled addressee was at the time the present invention was made. The filing date of the present application is 18th December 2000. Work leading to the patent application was completed in 1999. We are attaching copies of two review articles published in the 1990s which reflect the prevailing thinking of the time as to the role CRP plays *in vivo*. The first review is by Kilpatrick and Volanakis in Immunol. Res. (1991) 10:43-53. The second review is by Steel and Whitehead in Immunology Today (1994) 15:81-89. Both of these reviews indicate that **at the time the invention was made, CRP was generally regarded by persons of skill in the art as being beneficial for health.**

In the Kilpatrick review, first paragraph, CRP is categorized as an acute-phase protein whose plasma concentration increases during infection and inflammation. In the following paragraph, five lines from the bottom, it is indicated that it is generally believed that the acute-phase response is beneficial to the host. On page 46, under "Function of Human CRP," the review indicates that *in vitro* and *in vivo* experiments give results from which it is proposed that the function of CRP relates to its ability to specifically recognize foreign pathogens and damage to cells of the host and to initiate their elimination. In the following section the binding specificities of CRP are discussed and the binding of CRP to various ligands is described. In this section on page 47, 13 lines from the bottom of the first column, it is indicated that it has been proposed that CRP reacts with these components in damaged tissues and aids in their removal through interactions with the complement system and cells of the phagocytic system. The components include histones H1, H2A and H2B. In the next section on complement activation by CRP complexes, the sentence bridging pages 47 and 48 indicates that CRP-initiated activation of the classical pathway of complement leads to the assembly of an effective C3-convertase and it seems reasonable to assume that it results in the

generation of host defense-related complement fragments. In the following section on page 48, the opsonic properties of CRP are discussed. From line 16 of this section it is indicated that CRP enhances the phagocytosis of a variety of gram-positive and gram-negative pathogens. In the final paragraph of this section it is indicated that human CRP was shown to protect mice against fatal infection with type III and type IV *S. pneumoniae*. Additional studies indicated that not only human but also rabbit CRP could mediate blood clearance of *Pneumococci* in mice. On page 49 in the section entitled "Cellular receptors for CRP" the last paragraph indicates that CRP binds the phagocytic cells in a specific and reversible manner and that upon binding a biological response in the form of a phagocytic signal is elicited. The final section relates to CRP and platelet-activating factor. In the first sentence of this section it is indicated that, in addition to its role as an opsonin, CRP has been postulated to play a protective role in the early stages of an inflammatory reaction by inhibiting platelet-activating factor. At six lines from the bottom of this page it is indicated that the studies show that CRP protected neutrophils and platelets from the lytic effects of lysolecithin. In the final sentence it is indicated that, in addition to its role in host-defence and recognition and elimination of damaged cells, CRP also plays a role in control of the inflammatory response.

Taken together, these passages from the review by Kilpatrick and Volanakis show that prior to the discovery of the present application, **CRP was considered by persons of skill in the art to be beneficial to human health and to act in the acute-phase response beneficially against infection and inflammation.**

Turning now to the review by Steel and Whitehead, similar conclusions may be drawn. This review begins by confirming the acute phase of the inflammatory response to refer to the wide ranging physiological changes that are initiated immediately after an infection or physical trauma has occurred. A section on page 83 discusses CRP and SAP function and gene structure. CRP is a member of pentraxin family and in the last sentence to the first paragraph of this section it is indicated that the ability to bind to and to effect the clearance of nuclear material released from necrotic tissue during inflammation would provide an efficient means of preventing the initiation of nuclear-antigen specific autoimmunity and would explain at least in part, the need for high levels of circulating

pentraxins during inflammation. In the penultimate paragraph of this section, CRP is indicated to act as an opsinin for bacteria, parasites and immune complexes, and it can activate the classical pathway of complement. The activities of CRP are summarised in Figure 2. These activities confirm those discussed in the review by Kilpatrick and Volanakis and include opsonisation, complement activation, enhancement of phagocytosis of neutrophils and macrophages, enhancement of natural-killer activity and modulation of platelet activation. **Again, these are all activities recognised to be beneficial to the body when dealing with the acute phase of the inflammatory response.** On page 86 of this review under "The clinical significance of major APR induction" the exquisite responsiveness of CRP to acute phase stimuli, along with its wide concentration range and ease of measurement, have led to plasma CRP levels being used to monitor accurately the severity of inflammation and the efficacy of disease management during an infection. It is indicated that the ability of CRP to bind the microbial cell-wall components, as well as microbial and host chromatin, implies a role for CRP in clearing such cellular debris from the circulation.

This review by Steel and Whitehead, like the review by Kilpatrick and Volanakis, indicates, in light of *in vivo* data in particular, CRP is beneficial to health and beneficial in the response of the body to inflammation and infection.

It is submitted that reviews of this nature would typically form the general knowledge of one of ordinary skill in this technical field. **Accordingly, at the time the invention was made, a person of ordinary skill in the art would have recognized that the prevailing general knowledge based on *in vivo* experimental results was that CRP is a beneficial protein in the body's defense systems against inflammation.**

The reference of Bhakdi *et al.* describes *in vitro* experiments that quantify CRP binding to LDL and activation of complement, and hypothesizes that activation of complement by CRP in an atherosclerotic lesion may have pathological consequences. The question is whether a person of ordinary skill in the art, when reading Bhakdi, would have been motivated by this teaching to expect that inhibition of CRP binding to its ligands is a valid therapeutic target *in vivo* in the treatment of atherosclerosis or other inflammatory and/or tissue damaging conditions. We submit that **this speculative *in vitro* work does not**

provide appropriate incentive to one of ordinary skill in the art to perform the meth d of the claimed invention with a reasonable expectation of success. The documents cited by the Examiner fail to demonstrate that the interaction of CRP with LDL is relevant *in vivo*. Moreover, the observation that occurs *in vitro* does not permit one to predict whether this is a pathenogenetic interaction *in vivo*. Based on the *in vitro* observations alone, one of ordinary skill in the art could have concluded that the interaction could very well be beneficial. One might even speculate that it is atheroprotective. The potential for CRP as a therapeutic target would not have been validated by Bhakdi at all, and **the speculations of the Bhakdi reference would not have provided one of ordinary skill in the art with a reasonable expectation of successfully treating or preventing atherosclerosis in a living subject.**.

Importantly, in the light of the common general knowledge described above, it would have been completely counterintuitive for the skilled addressee to consider Bhakdi's *in vitro* findings to demonstrate CRP as a valid therapeutic target. Overwhelmingly, the common general knowledge based on *in vivo* data demonstrated that CRP is beneficial. Accordingly, it would not have been obvious to the skilled reader to apply Bhakdi in the way the Examiner suggests because Bhakdi does not provide convincing *in vivo* data sufficient to overturn the prevailing common general knowledge. Without a demonstration *in vivo* that CRP actually does increase tissue damage in a living animal, one of ordinary skill in the art would not have been motivated to arrive at the present invention.

Turning now to Kitao, this reference provides brief details of work in an abstract. Again, *in vitro* studies show binding of CRP to LDL. This binding could be beneficial *in vivo*. The abstract does not suggest inhibition of CRP binding to its ligands. No *in vivo* data are provided, either. This reference therefore does not remedy the deficiencies of the Bhakdi publication. Accordingly, we submit that it would not have been obvious to the skilled person to have arrived at the present invention from Kitao in combination with Bhakdi. To do so would have involved an inventive step because Kitao simply provides limited *in vitro* data concerning CRP binding to LDL which would not suggest to the skilled addressee that CRP was a realistic valid therapeutic target in the treatment or prevention of atherosclerosis or other tissue-damaging or inflammatory condition. It would have been completely

counterintuitive to have arrived at this conclusion given the c mmon general knowledge and *in vivo* evidence prevailing at the time.

Turning now to the secondary references applied by the Examiner, Yedgar is a US patent dealing with inhibitors of phospholipase A_2 activity. It is respectfully submitted that Yedgar has no bearing on CRP, Kitao or Bhakdi, and there is no reason for the skilled addressee to link Yedgar to Kitao or Bhakdi. As noted, Yedgar provides an extensive list of compounds that are PLA_2-inhibitors. Atherosclerosis is a very complex and incompletely understood process. The possibility that PLA_2 activity might contribute in some way to atherosclerosis has no bearing on the role played by CRP. One of ordinary skill in the art would therefore be unlikely to connect the inhibitors of PLA_2 described in Yedgar with the teachings of Bhakdi or Kitao to find motivation to make or use inhibitors of the binding of CRP to its ligands according to the present invention.

Turning now to Wissner, this document relates to phosphocholine derivates having antihypertensive action. Once again, there is no reason why one of ordinary skill in the art would turn to Wissner in the light of either Bhakdi or Kitao. Even if hypertension contributes in some way to atherosclerosis, this has no bearing on the role CRP might play. It is notable that the present application is concerned with tissue damaging conditions and not with drugs having antihypertensive action. Any connection between Wissner and either Bhakdi or Kitao appears to arise solely as a result of a hindsight reconstruction of the present invention.

The contribution made by the present inventor resides in a recognition that, in contrast to the prevailing common general knowledge at the time the invention was made, CRP markedly enhances tissue damage in an *in vivo* experiment involving acute myocardial infarction. This work clearly and definitively identifies CRP as a therapeutic target based on the first demonstration that human CRP actually causes increased tissue damage *in vivo* in a subject having an inflammatory/tissue-damaging condition. Contrary to the prevailing general knowledge, **the present invention comprises the first clear, direct demonstration of CRP causing more severe tissue damage than would have occurred in its absence,** with adverse clinical consequences *in vivo* both in myocardial infarction and cerebral infarction. The present invention thereby validates for the first time the inhibition of CRP binding to its ligands as a therapeutic strategy. Accordingly, whilst the prevailing common

general knowledge indicated that inhibition of CRP ligand binding activity should be harmful, the present inventor has demonstrated that such inhibition would be useful in a method of treating or preventing tissue damage in a subject having an inflammatory and/or tissue damaging condition.

The claimed invention is based on an unexpected finding and is submitted to be ground-breaking work. The present application demonstrates *in vivo* that CRP is a pathogenetic factor causing tissue damage and provides a range of inhibitors for treating or preventing such damage. As discussed above, the cited prior art references, taken in combination, would not have motivated one of skill in the art to administer a compound that inhibits the binding of CRP to its ligand to a subject with a reasonable expectation of successfully treating or preventing atherosclerosis and its complications. Prior to the pioneering discovery of the present application, one of skill in the art simply had no reasonable expectation that administration of a compound capable of inhibiting the binding of CRP to its ligand would successfully treat or prevent tissue damage in a subject having an inflammatory and/or tissue damaging condition such as atherosclerosis. Accordingly, the claimed method would not have been obvious to one of ordinary skill in the art in view of the cited references, and withdrawal of the rejection of the claims under 35 U.S.C. §103(a) is respectfully requested.

Conclusion

All rejections having been addressed, it is respectfully submitted that the present application is in condition for allowance and a Notice to that effect is earnestly solicited. If any points remain in issue, which the examiner feels may be best resolved through a personal or telephone interview, he is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,
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Attachments:

Copies of

(a) Steel et al., Immunology Today (1994) 15:81-89.

(b) Kilpatrick et al., Immunol. Res. (1991) 10:43-53.

(c) Form PTO-1449 citing the above two references.

(d) Medical Encyclopedia excerpt: Stroke secondary to atherosclerosis, from the "Medline Plus" portion of the website of the U.S. National Library of Medicine (URL: www.nlm.nih.gov/medlineplus/ency/article/000738.htm)

FORM PTO-1449 (modified)
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Examiner: Wang, S. Group Art Unit: 1617

U.S. PATENT DOCUMENTS

Examiner's Initials*		Document Number	Date MM/YYYY	Name (Family Name of First Inventor)	Class	Sub Class	Filing Date (if appropriate)

FOREIGN PATENT DOCUMENTS

		Document Number	Date MM/YYYY	Country	Inventor Name		English Abstract		Translation Readily Available	
							Enclosed	No	Enclose	No

OTHER (Including in this order Author, Title, Periodical Name, Date, Volume, Pertinent Pages, etc.)

	CCCCR	Steel et al., "The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein", Immunology Today (1994) 15:81-89.
	DDDDR	Kilpatrick et al., "Molecular Genetics, Structure and Function of C-reactive Protein," Immunol. Res. (1991) 10:43-53.

Examiner

Date Considered:

*EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communicati n to Applicant.

review

The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein

Diana M. Steel and Alexander S. Whitehead

Following an acute phase stimulus, such as infection or physical injury, many liver-derived plasma proteins are increased in concentration. These provide enhanced protection against invading micro-organisms, limit tissue damage and promote a rapid return to homeostasis. Diana Steel and Alexander Whitehead discuss the gene structure, regulation and possible clinical significance of the most dramatically induced acute phase reactants.

The acute phase of the inflammatory response refers to the wide ranging physiological changes that are initiated immediately after an infection or physical trauma has occurred. The mammalian acute phase response is characterized by fever, changes in vascular permeability, along with changes in the biosynthetic, metabolic and catabolic profiles of many organs[1]. The response is initiated and co-ordinated by a large number of diverse inflammatory mediators, which include cytokines, anaphylatoxins and glucocorticoids. Some of these are released initially at the site of inflammation by activated mononuclear phagocytes, lymphocytes or other differentiated cell types and have potent local and systemic effects. The ensuing cascade of mediators induces activation, proliferation, altered behaviour and changes in the biosynthetic capacities of a variety of target cells and tissues. This enhances host-survival by neutralizing the inflammatory agent and by promoting repair processes, thus initiating a return to normal function. It is important to consider the acute phase response (and inflammation) as a dynamic homeostatic process that involves all of the major systems of the body, in addition to the immune, cardiovascular and central nervous system (CNS). Normally, the acute phase response lasts only a few days; however, in cases of chronic or recurring inflammation, an aberrant continuation of some aspects of the acute phase response may contribute to the underlying tissue damage that accompanies the disease, and may also lead to further complications, for example cardiovascular disease or protein deposition diseases such as reactive amyloidosis.

An important aspect of the acute phase response is the radically altered biosynthetic profile of the liver. Under normal circumstances, the liver synthesizes a characteristic range of plasma proteins at steady state concentrations. Many of these proteins have important functions and higher plasma levels of these 'acute phase reactants' (APRs) are required during the acute phase response following an inflammatory stimulus. Although most APRs are synthesized by hepatocytes, some are synthesized by other cell types, including monocytes, endothelial cells, fibroblasts and adipocytes. So-called 'negative APRs' are decreased in plasma concentration during the acute phase response to allow an increase in the capacity of the liver to synthesize the induced APRs.

Inflammatory mediators that regulate APR synthesis

Of the many soluble factors that initiate and maintain an inflammatory response, several hormones specifically regulate the transcription of human APRs (Fig. 1). These include interleukin 1 (IL-1), IL-6, tumor necrosis factor α (TNF-α), leukemia inhibitory factor (LIF), transforming growth factor β (TGF-β), interferon γ (IFN-γ), glucocorticoids and the more recently identified effector molecules IL-11 (Ref. 2), oncostatin M (OSM) (Ref. 3), ciliary neurotrophic factor (CNTF) (Ref. 4) and retinoic acid[5]. In addition, insulin[6] and okadaic acid[7] have recently been shown to act as inhibitors of the cytokine-driven induction of some APRs. There is considerable heterogeneity in the response *in vivo* and *in vitro* of individual APR genes to the cytokines listed above (reviewed in Refs 8, 9). An important feature of the acute phase response is that IL-1 and TNF-α stimulate, *via* the CNS, the synthesis of glucocorticoids by the adrenal glands, which results in co-operative enhancement of the IL-1 and TNF-α-mediated induction of APR synthesis in the liver. This effect is coincident with the glucocorticoid-mediated downregulation of IL-1 synthesis by macrophages, thereby creating a negative-feedback loop between the immune and CNS systems to reduce *de novo* cytokine synthesis (Fig. 1).

Most of the increase (or decrease, in the case of negative APRs) in APR biosynthesis is due to increased (or decreased) gene transcription. This is mediated *via cis*-acting promoter elements that are binding sites for cytokine-activated (and, in some cases, induced) nuclear factors such as NF-κB, NF-IL-6/CAAT-enhancer binding protein β (C/EBPβ), NF-IL-6β/C/EBPδ, AP-1 and acute phase response factor (APRF), as well as for the glucocorticoid receptor and liver-specific transcription factors such as the hepatocyte nuclear factors (HNFs). However, there is increasing evidence for, and interest in, post-transcriptional events that may contribute to altered plasma levels of APRs. The demonstration of a TGF-β-mediated decrease in the half-life f Ap AI and

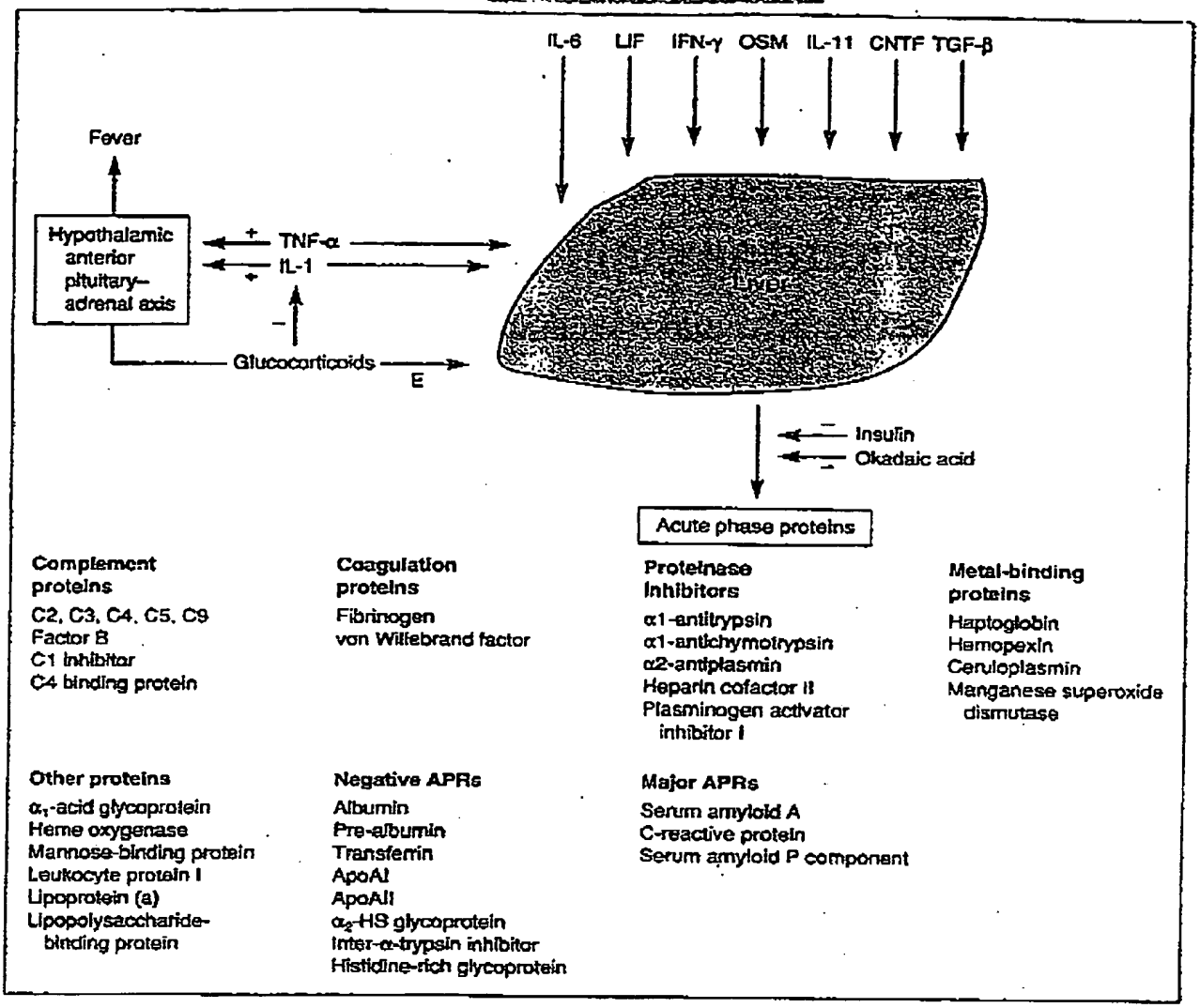

Fig. 1. Inflammatory mediators that modulate hepatic APR synthesis in humans. Abbreviations: (+), stimulation of activity; (–), inhibition of activity; (E), enhancement of activity; IL, interleukin; TNF-α, tumour necrosis factor α; LIF, leukemia inhibitory factor; IFN-γ, interferon γ; OSM, oncostatin M; CNTF, ciliary neurotrophic factor; TGF-β, transforming growth factor β; APR, acute phase reactant; ApoAI, apolipoprotein AI.

albumin mRNAs *in vitro* suggests one mechanism by which these negative APRs are downregulated[10]. Conversely, the stabilization of mRNA for positive APRs by cytokines and glucocorticoids, as well as the modulation of the polyadenylation of serum amyloid A protein (SAA) and α_1-acid glycoprotein (α_1-AGP) mRNAs during the acute phase response *in vivo* and *in vitro*[11,12] has been postulated to play a role in their upregulation. Cytokines may also regulate alterations in the glycosylation of some APRs with possible consequences for their physiological activity and stability[13].

Acute phase reactants have a wide range of activities

APRs have a wide range of activities that contribute to host defence[9,14–16] (Fig. 1): they can directly neutralize inflammatory agents, help to minimize the extent of local tissue damage, as well as participate in tissue repair and regeneration. There is a rapid increase in the plasma concentration of many complement cascade components the activation of which ultimately results in the local accumulation of neutrophils, macrophages and plasma proteins. These participate in the killing f infectious agents, the clearance of foreign and host cellular debris, and the repair of damaged tissue. Coagulation components, such as fibrinogen, play an essential role in promoting wound healing.

Proteinase inhibitors neutralize the lysosomal hydrolases released following the infiltration of activated macrophages and neutrophils, thus controlling the activity of the pro-inflammatory enzyme cascades mentioned above. The increased plasma levels of some metal-binding proteins help prevent iron loss during infection and injury, also minimizing the levels of heme iron available for uptake by bacteria and acting as

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scavengers for potentially damaging oxygen free radicals. Recent studies have shown that some mouse APRs are upregulated by heavy metals *in vivo*[17,18], which suggests that metal-binding proteins may themselves help to regulate the synthesis of other APRs. The changes in plasma concentrations of individual APRs are variable and probably reflect differences in the amount of each protein that is required to participate effectively in the acute phase response. Most APRs are induced between 50% and several-fold over normal levels. In contrast, the so-called 'major APRs' can increase to 1000-fold over normal levels. This group includes SAA and either C-reactive protein (CRP) in humans or its homologue in mice, serum amyloid P component (SAP).

The 'major APRs' CRP, SAP and SAA are massively induced

The major APRs in mammals include SAA and either CRP or SAP, depending on the species. Ironically, of all the APRs, the activities of these three are among the least well-known. Nevertheless, their interactions with other well-defined defence systems and the magnitude and rapidity of their induction following an acute phase stimulus, together with their short half-lifes[15], suggest a particularly critical requirement for these proteins very early in the establishment of host defence. Significantly, individuals unable to synthesize these proteins have not been described; these major APRs are therefore likely to be of considerable clinical importance. The *in vivo* and *in vitro* analyses of their dramatic induction in response to inflammatory mediators, along with their organization as gene families, serve as classic examples of how APR genes and their regulation have evolved.

CRP and SAP function and gene structure

CRP and SAP are pentraxins, proteins with a characteristic pentameric organization of identical subunits arranged as single and double annular pentagonal discs, respectively (reviewed in Ref. 19). CRP and SAP are found in all mammals and presumptive homologues have been found in a number of nonmammalian vertebrates and invertebrates. Generally, only one of these proteins is an APR in a given mammalian species: in humans, normal plasma SAP levels are approximately 30 $\mu g\ ml^{-1}$ and remain constant during inflammation but CRP levels can increase up to 1000-fold from approximately 1 $\mu g\ ml^{-1}$, depending on the disease and its severity. The species-specific induction of either SAP or CRP during the acute phase response suggests that the requirement for an acute phase pentraxin during inflammation is met by some common physiological activity, such as the capacity to bind chromatin[20,21]. The ability to bind to and to effect the clearance of nuclear material released from necrotic tissue during inflammation would provide an efficient means of preventing the initiation of nuclear-antigen specific autoimmunity and would explain, at least in part, the need for high levels of circulating pentraxins during inflammation.

In humans, CRP and SAP share only 51% amin acid identity and 59% nucleotide sequence identity. Both genes consist of two exons separated by an intron of 278 bp or 115 bp, respectively (Fig. 2): the first exon encodes a leader peptide and the first two amino acids of the mature protein, and the second exon encodes the remaining 202 (SAP) or 204 (CRP) amino acids. Interesting features of the transcribed portion of the CRP gene include an unusually long 3′ untranslated region (UTR), two heat-shock consensus sequences in the 5′UTR and a (GT)n region in the intron that, in theory, is capable of adopting a Z-DNA form and may play a role in gene regulation. CRP and SAP have remained in close physical and genetic linkage, mapping to syntenic regions in man and mouse: band q2.1 of chromosome 1 and the distal portion of chromosome 1, respectively. In both species many genes flanking this locus have immune and inflammation-related roles: many of the Fc receptors to date have been mapped to the same regions (reviewed in Ref. 22), as have a group of interferon-inducible products in mouse[23] and one in human (M.T. Walsh and A.S. Whitehead, unpublished). It is likely, therefore, that the entire region around the pentraxin genes will be of considerable scientific and clinical importance. Computer-based evolutionary analyses of cloned mammalian CRP and SAP genes indicate that they are rapidly evolving and probably duplicated from a common ancestral pentraxin gene as early as 200 million years ago, prior to the time of the divergence of eutherian mammals and marsupials[24]. A novel human pentraxin, PTX3/TSG-14, that shares limited homology with CRP and is induced in hepatocytes, endothelial cells and fibroblasts by IL-1β or TNF-α has recently been reported[24,25], indicating that additional members of this family are yet to be described.

CRP was originally named for its ability to bind the C-polysaccharide of *Pneumococcus* and has since been shown to have a number of calcium-dependent binding specificities (reviewed in Ref. 14) and biological activities related to nonspecific host defence (reviewed in Refs 19, 26). It acts as an opsonin for bacteria, parasites and immune complexes, and can activate the classical pathway of complement. Together, these functions can modulate the behavior of several cell types, including neutrophils, monocytes, natural killer cells and platelets (Fig. 2). In addition, as mentioned above, CRP can bind to chromatin, histones, and small nuclear ribonucleoprotein particles (snRNPs).

SAP is the circulating form of amyloid P (AP) component, which is a constituent of all types of amyloid deposits (reviewed in Ref. 27). It is a normal component of basement membranes, has a number of calcium-dependent binding capabilities and, similar to CRP, functions in nonspecific immunity (Fig. 2). In common with CRP, SAP has the capacity to bind chromatin and histones, as well as DNA (reviewed in Refs 27, 28) and can activate the classical complement pathway *via* binding to C1q (Ref. 29).

CRP and SAP biosynthesis during the acute phase response

The acute phase expression of CRP and SAP has been studied *in vivo* using animal models of inflammation and *in vitro* by treating primary hepatocyte or

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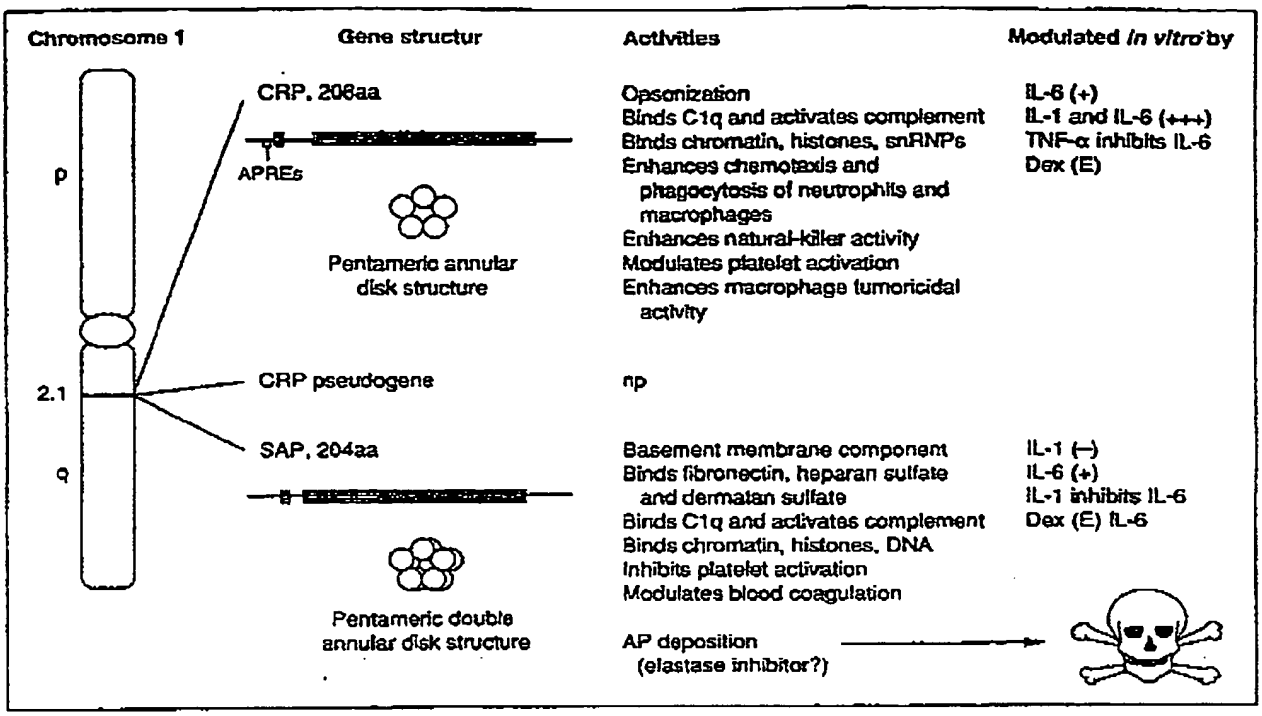

Fig. 2. *Gene structure, functions and regulation of the human pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP). Abbreviations: aa, amino acid; APRE, acute phase response element; np, no product; (+), (++), (+++), relative degree of induction; (–), inhibition; (+/–) can induce or inhibit; (E), enhances; IL, interleukin; TNF-α, tumour necrosis factor α; Dex, dexamethasone; snRNPs, small nuclear ribonucleoprotein particles; AP, amyloid P component.*

hepatoma cultures with inflammatory mediators. Mice given a single experimental inflammatory stimulus, for example *via* intraperitoneal injection of thioglycollate, show massive increases in hepatic SAP mRNA levels within two to four hours, with peak concentrations by 8–12 hours[30]. These are followed by elevated circulating SAP protein levels peaking around 24–36 hours. Background levels both of hepatic mRNA and plasma protein are re-established by 72 hours in accord with the transient nature of the acute phase response. *In vitro* studies of SAP synthesis in mouse hepatocyte cultures show that the addition of IL-1α causes a tenfold increase and the addition of IL-6 causes a sevenfold increase; the addition of both cytokines together has an additive effect[31].

Several *in vitro* studies of human CRP, using hepatoma cells, have shown that CRP mRNA transcription is induced dramatically by IL-6 (Refs 32–35) with protein synthesis being further regulated by translational and post-translational mechanisms. Although no significant change is elicited by IL-1β alone, when it is used in combination with IL-6 there is a greatly enhanced response[34,35]. The promoter of the human CRP gene contains two acute phase response elements (APRE). These are APRE1, which contains a binding site for the liver-specific transcription factor HNF1, and APRE2, which contains an HNF1 binding site (β site) and an NF-IL-6 (C/EBPβ) binding site (α site) (reviewed in Ref. 36). NF-IL-6 is a recently identified transcription factor, induced by IL-6 (Ref. 37) and activated by PKC-dependent phosphorylation at Ser105 (Ref. 38). It has the ability to interact with the promoter regions of several IL-6-inducible genes at a consensus sequence of TG(G/A)AA (Ref. 39). In short, IL-6 activates NF-IL-6, which interacts with the α site and enhances binding of HNF1 to the β site, resulting in the co-operative liver-specific induction of CRP. A number of additional proximal regulatory regions have been fine mapped[40]: an additional IL-6RE, two negative-control regions, two constitutive enhancer-like elements, as well as three additional distal elements upstream of nucleotide -292. The mouse CRP gene contains two HNF1-responsive elements that overlap with a C/EBP- and IL-1-responsive element; additional regulatory-element consensus sequences are present in the 5′ portion of the gene but they are apparently not functional[41]. CRP is also induced several fold in mice in response to heavy metals (Hg, Cd, Pb, Cu, Ni, Zn) and a putative metal-responsive element has been identified in the promoter of the mouse CRP gene[17,18].

In addition, an IL-1-responsive sequence between –42 and +15 has been identified in the human CRP gene, but this sequence was thought to act as a translational modulator[42]. Translational regulation by IL-1 has been demonstrated for the heavy and light chain mRNAs of ferritin[43], and is due to the presence of a *cis*-acting sequence motif, the so-called 'acute box', in the 5′UTR of ferritin mRNAs (J.T. Rogers, unpublished). A similar sequence is present in the 5′UTR of SAA and several other APR mRNAs and could function as an IL-1-responsive translation modulator. Although the CRP 5′UTR does not appear to contain the acute box

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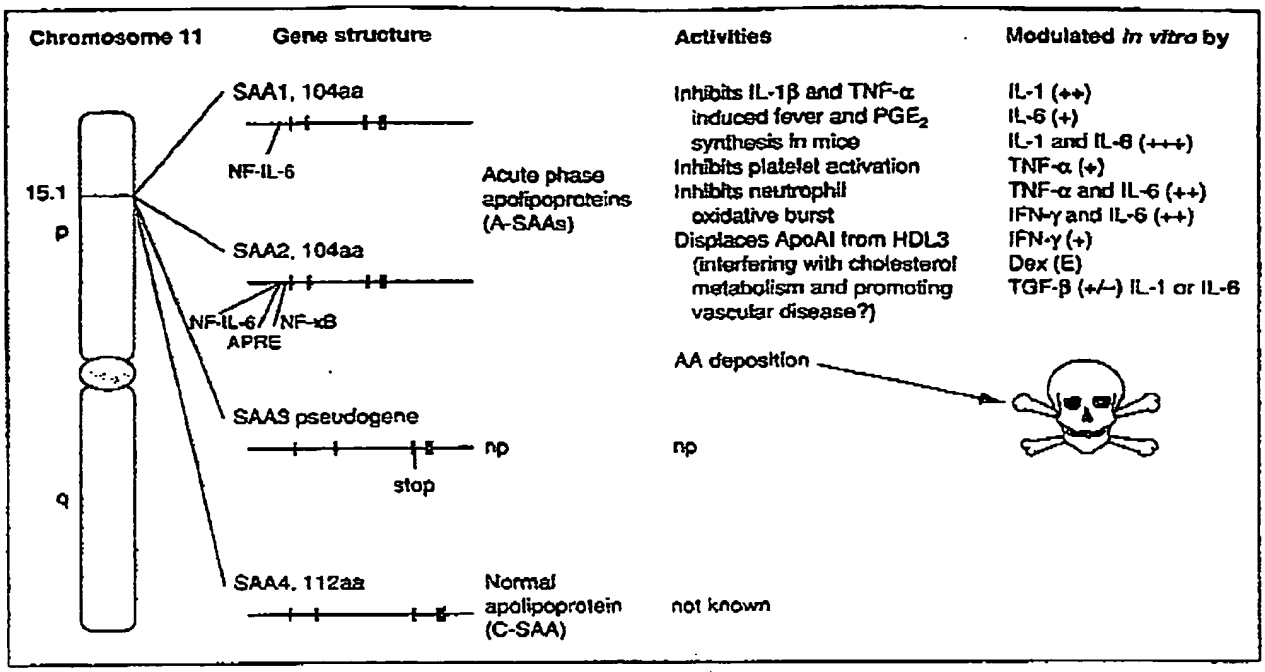

Fig. 3. *Gene structure, functions and regulation of the human serum amyloid A (SAA) gene family. Abbreviations: aa, amino acid; NF-IL-6, nuclear factor interleukin 6 (IL-6); APRE, acute phase response element; HDL, high-density lipoprotein; np, no product; TNF-α, tumour necrosis factor α; PGE_2, prostaglandin E_2; IFN-γ, interferon γ; Dex, dexamethasone; TGF-β, transforming growth factor β; (+), (++), (+++), relative degree of induction; (–), inhibition; (+/–), can induce or inhibit; (E), enhances; AA, amyloid A protein; ApoAI, apolipoprotein AI.*

consensus, it is possible that translational regulation of CRP by IL-1 is effected by a similar mechanism.

Post-translational control of CRP has also been demonstrated: the secretion of CRP by rabbit hepatocytes becomes more efficient during the acute phase reponse[45] due to the downregulation of a specific binding site by which CRP is normally retained within the endoplasmic reticulum (ER) under nonacute phase conditions[46]. CRP can be further autoregulated by the CRP-mediated release from neutrophils of a protease that promotes its own degradation[47]. Thus, the dramatic transcriptional induction of CRP by IL-6 is further enhanced by novel translational and post-translational mechanisms, which eventually lead to a negative-feedback loop in which CRP can downregulate its own levels during an inflammatory response.

Human SAP mRNA accumulation can also be induced by IL-6, however, treatment of hepatoma cells with IL-1β leads to downregulation of SAP mRNA synthesis, an effect that predominates when both cytokines are present[34]. A similar effect has been demonstrated for C1 inhibitor[34], suggesting that, although SAP and C1 inhibitor are not considered to be significant acute phase proteins in humans, there may be conditions, especially at local sites, under which the relative proportions of available cytokines would promote increased nonhepatic SAP or C1 inhibitor synthesis. The *cis*-acting promoter elements in the mouse SAP gene have been mapped[48]. However, the IL-6-responsive promoter elements and the *cis*-acting sequences and DNA-binding proteins responsible for the IL-1-mediated downregulation of the human SAP gene, are likely to behave quite differently.

Serum amyloid A superfamily gene structure

SAA is the collective name given to a family of polymorphic proteins encoded by multiple genes in a number of mammalian species. SAA genes have a common four exon/three intron organization that is characteristic of other apolipoprotein genes (Fig. 3). In humans, two acute phase SAA (A-SAA) genes have been described, *SAA1* and *SAA2*, which are almost identical with respect to the primary structures of their specified products, their gene organizations and sequences, and their mode of expression. They are undoubtedly the result of an ancestral gene duplication followed by regular gene conversion events. A third gene (*SAA3*) is a pseudogene and a fourth (*SAA4*) encodes 'constitutive SAA' (C-SAA), which is minimally induced during inflammation[49]. C-SAA shares only 55% identity with the SAA1 and SAA2 proteins and its expression is very different from that of the A-SAAs (reviewed in Ref. 50). The human SAA genes are clustered and all map to the short arm of chromosome 11 (Ref. 51). In mouse, two major acute phase SAA genes (*SAA1* and *SAA2*), a minor acute phase gene (*SAA3*), and an SAA pseudogene (*ψSAA/SAA4*) (Refs 52, 53) are closely linked on a syntenic region of chromosome 7 (Ref. 54). The product of a putative fifth mouse SAA gene ('*SAA5*') has also been described[55], though a homologue encoding C-SAA has not yet been identified in the mouse. It is likely that multiple SAA superfamily genes, displaying different degrees of relatedness and different induction characteristics, are clustered in other species.

Functionally, SAAs are small apolipoproteins that associate rapidly during the acute phase response with the third fraction of high-density lipoprotein (HDL3),

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on which they become the predominant apolipoprotein[56], exceeding ApoAI in quantity (Fig. 3). Although the functional significance of SAA association with HDL3 has not been formally established, the apparent displacement or replacement of ApoAI by SAA may interfere with cholesterol metabolism[57]. A recent study demonstrated that SAA enhances the binding of HDL3 to macrophages during inflammation, concomitant with a decrease in the binding capacity of HDL3 to hepatocytes[58]. These data support one hypothesis that SAA may remodel HDL3 and act as a signal to redirect it from hepatocytes to macrophages, which can then engulf cholesterol and lipid debris at sites of necrosis. Excess cholesterol could thus be redistributed for use in tissue repair or excreted.

Nevertheless an acute phase role(s) for the SAAs has not been definitely established and several alternative (or additional) functions have been proposed (Fig. 3). In mice, SAA has been shown to inhibit IL-1β and TNF-α-induced fever *in vivo* and PGE_2 synthesis in hypothalamic slices *in vitro*[59], which suggests a possible feedback relationship with IL-1β and TNF-α (both of which induce SAA transcription), involving the CNS. Other putative protective roles for SAA are the inhibition of thrombin-induced platelet activation[60], as well as inhibition of the oxidative burst in neutrophils, which would help prevent oxidative tissue destruction[61]. Paradoxically, rabbit SAA3 induces collagenase synthesis in rabbit synovial fibroblasts, suggesting an autocrine regulatory role for this superfamily product at local inflammatory sites[62,63].

SAA biosynthesis is regulated transcriptionally and post-transcriptionally

The differential expression of SAA superfamily members constitutes one of the best systems for the study of *cis*-acting regulatory mechanisms in APR genes and their products. Different human hepatoma-cell lines differ in their abilities to synthesize SAA in response to individual cytokines; in general, IL-1β and IL-6 can both induce SAA mRNA and protein synthesis, though the effect of IL-1β is substantially more potent than IL-6. However, when used together, these cytokines show a dramatic synergistic induction. Our studies with PLC/PRF/5 cells have confirmed that the principal means of exercising control of SAA expression following an inflammatory stimulus is *via* increased transcription and the accumulation of mRNA[10]. However, when induced SAA mRNA levels are at their peak, synthesis of SAA protein is less than would be expected from a strictly proportional use of the accumulated mRNA when compared with earlier times post-stimulus, suggesting modulation at the translational level. This modulation does not appear to be due to progressive variation in the A-SAA export capacities of the cells at different times post-stimulus, as is the case for CRP (Ref. 45), or differential engagement of A-SAA mRNA by the cellular translation apparatus (i.e. ribonucleoproteins, monosomes and polysomes), as is the case for ferritin heavy- and light-chain mRNA[45]. Although the cellular levels of cytokine-induced A-SAA mRNA decrease rapidly after reaching their peak, A-SAA mRNA in actinomycin D-treated and cycloheximide-treated cells has a half-life of >24 hours, indicating that under those conditions the mRNA is very stable. This suggests the existence of an active mRNA degradation process that is dependent on *de novo* mRNA and/or protein synthesis, which commences at some point after cellular induction by cytokines.

Current efforts to fully characterize the factors contributing to the biosynthesis of human A-SAA are focused on the mRNA poly(A) tail, which shows a controlled and progressive shortening after the appearance of induced mRNA (at about 3 hours) to 72 hours post-cytokine stimulus[11]. Poly(A) tail shortening has also been demonstrated for SAA mRNA *in vivo* in animals undergoing experimental inflammation and *in vitro* for the mRNA specifying another acute phase reactant, α_1-AGP. This process may be regulated by inflammatory mediators in order to effect specific mRNA processing events and/or to regulate the efficiency of translation.

By contrast, C-SAA (the product of the *SAA4* gene) is minimally induced by inflammatory cytokines *in vitro* (D.M. Steel, unpublished). Although its genetic organization is largely the same as that of the A-SAA genes, the sequence and motifs contained in its promoter region and introns are very different[50]. The human *SAA2* promoter region contains a NF-κB binding site[64]. A cytokine-responsive element that probably binds NF-κB (Ref. 65) and another *cis*-acting element that binds to the liver-specific transcription factor C/EBP have been identified in the mouse *SAA3* gene[66] and the rat *SAA1* gene[67]. A comparative analysis of human *SAA1*, *SAA2* and *SAA4* promoter activity will be useful in further defining the intrinsic genetic elements required for transcriptional activation of these genes in the liver and other organs.

The clinical significance of major APR induction

The exquisite responsiveness of CRP to acute phase stimuli, along with it's wide concentration range and ease of measurement, have led to plasma CRP levels being used to monitor accurately the severity of inflammation and the efficacy of disease management during an infection (reviewed in Ref. 68). The ability of CRP to bind the microbial cell-wall components, as well as microbial and host chromatin, implies a role for CRP in clearing such cellular debris from the circulation. Conversely, some diseases (eg. systemic lupus erythematosis) are associated with relatively low plasma levels of CRP (Ref. 69).

SAA and SAP are archetypal examples of plasma proteins that are likely to be beneficial during the transient acute phase response but which have detrimental effects in chronic inflammation. These major APRs have been implicated in a number of clinical conditions. Secondary, or reactive, amyloidosis is the occasional consequence of a variety of chronic and recurrent inflammatory diseases[27], for example leprosy, tuberculosis, systemic lupus erythematosis and rheumatoid arthritis. It is characterized by the ultimately fatal deposition of insoluble fibrils in a number of tissues, including spleen, liver and kidney. Secondary amyloid deposits are composed mainly of amyloid A (AA) derived (probably by proteolysis) from the

precursor SAA (Ref. 70), and are deposited as β-pleated sheets. Amyloid P component (AP), derived from SAP, is associated with secondary AA plaques and all other forms of amyloid deposits, including those present in Alzheimer's disease[27]. AP has also been shown to act as an elastase inhibitor *in vitro*[71,72], which would suggest a role for SAP on amyloid deposits in protecting the fibrils from degradation by proteolytic enzymes. Alternatively, given its multimeric configuration and binding characteristics, SAP may act as a nucleating agent in fibril deposition. Although not a major APR in humans, SAP may be induced locally or systemically by different combinations of cytokines. It may, therefore, be clinically important to devise strategies that can specifically downregulate SAA and SAP during chronic inflammation, in order to inhibit the progression of amyloid deposition by reducing their concentrations to an 'amyloidogenic threshold' below which clearance processes can predominate.

The association of SAA with HDL3 suggests another area in which chronically high SAA concentrations may promote clinical disease. HDL is central to reverse cholesterol transport, and there is a significant decrease in plasma HDL cholesterol during inflammation[56]. As mentioned above, during inflammation the association of A-SAA with HDL3 modifies the particle and may equip it for a protective host-defence role for which there is an overriding short term need. High SAA levels on HDL3 are achieved at the expense of ApoAI, a critical activator for the lecithin-cholesterol acyltransferase reaction. By displacing ApoAI (directly or indirectly) from the HDL particle, A-SAA may diminish the functional capacity of HDL to mediate reverse cholesterol transport. In association with the sustained decrease in total HDL that takes place during the acute phase, this would constitute a major risk factor for the development of atherosclerosis in individuals with chronic recurrent inflammation. This could provide a molecular explanation for the increased mortality from cardiovascular disease observed in patients with reactive systemic rheumatoid arthritis.

In addition, evidence that locally produced SAA3 in rabbits can act as a collagenase inducer[43] implies that increased levels of some SAA isoforms not only result in secondary complications of inflammatory diseases, but that they may play an active role in the pathogenesis of the inflammatory diseases themselves. A thorough examination of the structure, expression, and molecular genetics of all of the members of the SAA superfamily is likely, therefore, to be of considerable clinical, as well as biological, importance.

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letters

Surface-bound cytokines – a possible effector mechanism in bacterial immunity?

Cytokines are generally thought of as acting as soluble mediators; however there is an increasing body of evidence that suggests that immobilization of cytokines on solid surfaces may be important in regulating several aspects of the immune response[1–4]. In particular, a number of cytokines are able to bind to glycosaminoglycan (GAG) side-chains carried by proteoglycans on cell surfaces[3,4] and may provide important haptotatic and localization signals[1–4]. These cytokines include the chemokine or intercrine family of low-molecular-weight factors, as well as other factors of higher molecular weight, such as interleukin 7 (IL-7), granulocyte–macrophage colony-stimulating factor (GM-CSF) and transforming growth factor β (TGF-β). Indeed, it has been shown that the chemokine macrophage inflammatory protein 1β (MIP-1β) will bind to proteoglycans on the surface of endothelial cells and trigger T cells to express functionally active, adhesive integrins[4]. I would like to suggest that a similar mechanism may be important in bacterial immunity, and that some chemokines and other cytokines could bind to sugar residues on the surface of bacterial cells, thereby promoting their killing by leukocytes.

Chemokines have a wide range of effects on leukocytes. Prominent among such effects is chemotaxis and promotion of adherence – functions that are related to their role in the recruitment of cells to inflammatory sites[5]. However, in addition, some members of the chemokine family are able to induce cytotoxic mechanisms. For example, IL-8 and MIP-1α and -β can induce neutrophils to degranulate, release lysosomal enzymes and produce reactive oxygen species[5,6]. Chemokines such as IL-8 and macrophage chemotactic and activating factor (MCAF) can stimulate cytotoxic mechanisms in macrophages and induce histamine release by basophils[5]. Thus, if such cytokines were immobilized on the surface of bacteria, they could provide a powerful stimulus both for the adherence (and possible phagocytosis) of the bacteria by macrophages and granulocytes, and for the induction of cytotoxicity by those cells.

A number of carbohydrates exist n the surface of bacteria that might serve to anchor soluble factors; these include peptidoglycan

(exposed on the surface of Gram-positive bacteria) and a wide variety of polysaccharides. Peptidoglycan comprises chains of alternate *N*-acetyl-D-glucosamine and *N*-acetyl-D-muramic acid crosslinked by a tetrapeptide. The polysaccharides are very diverse, but can incorporate hyaluronic acid and sulphated sugars that are similar to those seen in the GAG side-chains of proteoglycan[7]. Indeed, the K5 capsule of *E. coli* has an identical sequence to that found in heparin[8]. As GAG-binding cytokines are not indiscriminate in their ability to bind to GAG (Ref. 9), but are capable of recognizing specific structures, it would appear likely that some of these molecules may be adapted to recognize sugar residues on the surface of bacteria, leading to the coating of the pathogen with soluble factors. This hypothesis is supported by the finding that bacterially derived D-gluco-D-galactan sulphate species bind to basic fibroblast growth factor (bFGF) (Ref. 10). This growth factor binds to both high- and low-affinity sites ('heparin-like') on endothelial cells. Binding to the low-affinity site can be blocked with heparin (but not with chondroitin sulphate), whilst binding to both receptors can be blocked with D-gluco-galactan sulphate, suggesting that it has a high affinity for the growth factor[10].

The binding of soluble cytokines onto bacterial cell-surfaces, leading to the formation of a multivalent array of solid-phase mediators is, potentially, a very important pathway of leukocyte recognition and activation. Cytokines bound to the surface sugars of bacteria could provide opsonin-like signals that mediate the attachment and phagocytosis of the pathogens by effector cells, and also help trigger leukocyte cytotoxic mechanisms.

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Dynamics of the autoimmune T-cell repertoire in experimental allergic encephalomyelitis and in multiple sclerosis

In the May issue of *Immunology Today*, Lehmann *et al.*[1] review data that provides evidence for the 'spreading of myelin basic protein (MBP) determinants' in the T-cell response during experimental allergic encephalomyelitis (EAE). They propose that similar dynamics may be an important event in the pathogenesis of multiple sclerosis (MS) and other organ-specific autoimmune diseases.

Recently, we have approached this problem directly in MS by screening the fine specificity of numerous MBP-specific T-cell lines in single patients with MS at different time points during the course of the disease[2]. Patients could be divided into two groups, one with T-cell responses to several MBP epitopes, the other with restricted patterns of reactivity to single regions of the molecule (similar results have also been obtained in other laboratories: H. Wekerle and K. Wucherpfennig, pers. commun.). The differences in the two groups could not be explained by clinical features, such as disease duration, phase, severity and magnetic resonance imaging (MRI) findings. The only distinctive feature was that a common HLA-DR2 serological specificity was observed in patients with restricted T-cell responses and that these responses appeared to be stable over time.

On the strength of these observations, we would like to suggest the following: (1) Responses that are restricted to specific regions of MBP may be of pathogenetic relevance in some patients with MS. In two cases where we observed such responses, the T-cell reactivity was directed against an epitope (residues 86–99) located within a region found to be encephalitogenic in the Lewis rat. This epitope recalls more frequent T-cell responses in MS patients than in healthy individuals[3]. Moreover, when we tested MBP-specific T-lymphocyte lines from three HLA-DR2-matched donors that did not have MS, we detected that the responses were against several MBP epitopes. However, our approach did not allow a clear definition of the pathogenetic relevance of cryptic epitopes: we drove the T-cell lines using intact MBP, rather than peptides, and therefore probably precluded the detection of a cryptic repertoire. However, in a recent study, Satyanarayana *et al.*[4] were unable to determine differences between MS patients and controls in the frequency of T cells specific for cryptic epitopes. Since it is possible that cryptic sequences were not represented by the peptide panel used in this study, the relevance of cryptic MBP epitopes in MS remains unclear.

(2) The genetic background of patients seems to influence the

(10)

C-Reactive Protein

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Molecular Genetics, Structure, and Function of C-Reactive Protein

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Human C-reactive protein (CRP) can be classified under two partially overlapping groups of proteins. On the basis of its best-known biological property, i.e., the striking increase of its plasma concentration during infection and inflammation, CRP is categorized as an acute-phase protein. On the basis of its structure and Ca^{++}-dependent binding specificities, CRP is classified as a pentraxin.

Acute-phase proteins constitute a heterogeneous group of proteins of hepatic origin that share the property of increased plasma concentration during infection and/or tissue injury [1]. CRP is the most characteristic human acute-phase protein, since its plasma concentration rises by several hundredfold within 24–48 h from tissue injury. These high levels persist for the duration of the acute-phase response, returning to the normal low concentrations with restoration of tissue structure and function. It is generally believed that the acute-phase response is beneficial to the host, although the potential contributions of certain acute-phase proteins to homeostasis and self defense are not self-evident.

Pentraxins (from the Greek πέντε, five and ῥάξ, berry) [2] constitute a phylogenetically ancient family of proteins exhibiting a remarkable conservation of structure and binding reactivities. The most ancient member of the family is the major protein of the hemolymph of the invertebrate *Limulus polyphemus* (horshoe crab) [3]. Members of the pentraxin family have also been found in the blood of the dogfish, *Mustelus canis* [4], the blood of certain marine teleosts [5, 6] and of all vertebrates examined [7]. All pentraxins consist of single polypeptide chain subunits, arranged in pentagonal or rarely hexagonal cyclic symmetry and discernible by electron microscopy. Comparison of the primary structures of pentraxins from several species indicates that *Limulus* CRP exhibits approximately 25% amino acid residue identity to mammalian pentraxins. Among the latter, identity ranges from 50 to 75% [8]. Two regions, each approximately 15 residues long, with highly conserved amino acid sequence have also been identified in all pentraxins [9].

All pentraxins bind Ca ions which are necessary for the expression of ligand-bind-

ing activity. In vertebrates there are two main branches of the pentraxin family. CRP-like members bind phosphocholine (PCh), while serum amyloid P-like members bind carbohydrate moieties. Many animal species have both CRP and serum amyloid P, but in some species both binding specificities reside in a single molecule [3]. Not all pentraxins are acute-phase proteins. For example, in humans and rabbits, CRP is an acute-phase protein and serum amyloid P is not, while in mice the reverse is true [10]. The stable conservation of structure and binding specificities, through an extremely long evolutionary time estimated at more than 500 million years, suggests strongly an important biologic function for pentraxins.

Structure of the Human CRP Gene

The single copy of the human CRP gene is located on the proximal arm of chromosome 1 [11, 12]. It spans approximately 2.5 kbp of DNA and is located 7.7 kbp upstream of a CRP pseudogene [13 14]. The gene consists of two exons separated by a single, 278 bp long, intron. The first exon encodes a putative signal peptide consisting of 18 amino acids and the first two amino acids of the mature protein. The remaining 204 amino acids of the polypeptide chain and a 1.2 kbp 3′ untranslated region are encoded by the second exon [15, 16]. Primer extension experiments indicated that the mRNA cap site is located 104 nucleotides upstream of the initiation codon. A typical promoter region, consisting of a TATA box and a CAAT box located 29 and 81 nucleotides, respectively, upstream of the cap site, has been identified [15, 16]. Northern blotting indicated a 2.2-kbp length for the CRP mRNA [17].

The nucleotide sequence of the single CRP gene intron is interesting in that it contains two characteristic repetitive elements: a run of 16 adenines is located 77 nucleotides into the intron and a repeating purine-pyrimidine (GT) stretch is present 33 nucleotides downstream [16]. The number of GT repeats exhibits polymorphic variation with three alleles containing 15, 19, or 22 repeats described [13]. It has been pointed out that this region could adopt the Z-form of DNA and thus, could play a role in chromatin activation.

The dramatic rise of the plasma CRP concentration following insult or injury and the abrupt return to normal levels after restoration of normal tissue structure have stimulated a considerable amount of research aimed at defining the cis-acting regulatory elements of the CRP gene. Initial comparisons of the sequence of the 5′ flanking region of the CRP gene to known regulatory sequences indicated the presence of three areas homologous to the *Drosophila* heat shock consensus sequence [15]. These areas are located at nucleotide −146 to −134, −130 to −119, and −95 to −86. However, the functional significance of these elements remains unknown. Ciliberto et al. [18] have taken advantage of the differences in expression of CRP in mice and humans to analyze the expression of human CRP in transgenic mice. Unlike human CRP, mouse CRP is not an acute-phase reactant [10]. The investigators introduced a 30-kbp human DNA fragment containing the CRP gene into the genome of C57/BL6XSJL mice. The CRP gene in this fragment was flanked by 16 kbp of DNA at the 5′ end and by 10 kbp at the 3′ end. Human CRP was expressed by transgenic mice only upon induction of inflammation by administration of endotoxin. The

response was tissue-specific with transcription occurring exclusively in the liver. Human CRP mRNA was detected 2 h after stimulation and the protein was detectable in the serum 4 h later. Levels of CRP in the sera of the transgenic mice were comparable to those observed in human diseases. Nuclear run-on experiments indicated that control was exercised mainly at the transcriptional level. These experiments established that cis-acting regulatory elements of the human CRP gene are responsible for both tissue specificity and acute-phase inducibility of CRP expression and that the trans-acting factors involved are conserved throughout evolution for mouse to man.

Arcone et al. [19] utilized an in vitro system to analyze cis-acting regulatory elements in the 5'-flanking region of the human CRP gene. Constructs containing this region and the chloramphenicol transferase gene were transfected into mammalian cells. Stimulation of the transfected cells with monocyte-conditioned media resulted in expression of the reporter gene only in hepatocytes but not in HeLa cells, indicating that elements responsible for acute-phase inducibility and tissue-specificity of CRP expression are within 2.5 kbp of 5'-flanking DNA from the CRP gene. 5'- and 3'- deletions of the flanking region revealed the presence of two inducible elements: the first between nucleotides –94 and –50 and the second upstream from this region with its 3' boundary between positions –106 and –137. A more detailed study of the cis-acting regulatory elements of the CRP gene was reported more recently [20]. Using 5' flanking region chloramphenicol transferase constructs, transfection into the human hepatoma cell line PLC/PRF/5, and recombinant interleukin-6 (IL-6) as inducer, the authors defined two IL-6-inducible elements. The first, located between –86 and –60, is probably identical to the proximal element identified by Arcone et al. [19], the second is located upstream between –234 and –200. The two elements can function independently of each other and contain the consensus motif, TG (G/A) AAAA. This motif is present in three copies in the distal IL-6-inducible element and in one copy in the proximal one and is similar to that found in the rabbit CRP promoter. In addition, this motif is discernible in the IL-6-responsive elements present in the genes encoding the acute-phase proteins, human haptoglobin and rat α_1-acid glycoprotein. Additionally, two constitutive enhancer-like elements and a strong negative element were delineated within the 900-bp 5'-flanking region of the CRP gene.

Structure of Human CRP

CRP is made up of five identical, noncovalently bound subunits [21], exhibiting a planar pentagonal appearance in electron micrographs [2]. A molecular weight of 118,000 has been calculated for the native pentameric molecule from sedimentation equilibrium studies [22]. Each subunit consists of 206 amino acid residues with a calculated molecular weight of 23,017 [14, 15]. A single disulfide bond links the two half-cystines at positions 36 and 98 [15, 16, 23]. There are no potential N-glycosylation sites in the amino acid sequence and no carbohydrate is present in purified CRP. Two different crystal forms of human CRP have been grown from solutions of 2-methyl-2,4-pentanediol. Both forms are tetragonal and their unit cell parameters have been reported [24]. The crystals of form II diffract to 3.0 Å and

are suitable for structural studies. X-ray diffraction studies have yielded a solution of the rotation function, confirming the pentameric structure of the molecule.

Function of Human CRP

On the basis of results of in vitro and in vivo experiments, it has been proposed [25, 26] that the function of CRP relates to its ability to specifically recognize foreign pathogens and damaged cells of the host and to initiate their elimination by interacting with humoral and cellular effector systems in the blood. This proposal assigns a recognition and an effector function to the CRP molecule. These functions will be reviewed briefly below.

Binding Specificities of CRP

The first described reactivity of CRP which led to its discovery and naming, was for the C-polysaccharide of the cell wall of pneumococci (PnC) [27]. The reaction resulted in precipitation but it could be differentiated from antigen-antibody reactions in that it required Ca ions. Studies on the structure of PnC demonstrating its ribitol teichoic acid nature [28] led to the demonstration that phosphate groups on PnC constitute the main determinants recognized by CRP [29]. A number of phosphate monoesters were shown to bind to CRP with binding constants of $2-3 \times 10^4\ M^{-1}$ and a valence of one per subunit [29]. This binding required Ca^{++} and it was also shown that Ca^{++} bound to CRP with a constant of $10^4\ M^{-1}$ and a valence of one or two per subunit. More recently, it was shown that binding of Ca^{++} induces a conformational change in the CRP molecule, which can be demonstrated by changes in the aromatic region of the CD spectrum of the molecule [30] and also immunochemically [31]. It was subsequently shown that PCh is a far more potent inhibitor of the CRP-PnC precipitation reaction than other phosphate monoesters [32], which led to the proposal that PCh residues on PnC provide the major determinant group for the binding of CRP. Equilibrium dialysis experiments indicated that in the presence of Ca^{++}, CRP binds PCh with an association constant of $1.6 \times 10^5\ M^{-1}$ [33] and a valence of one per noncovalent subunit. The specificity of CRP for PCh is further supported by results indicating binding of the protein to emulsions containing phosphatidyl choline (lecithin) [34] and to other cell wall polysaccharides containing PCh [35]. In addition, CRP was shown to bind to lecithin liposomes and single-walled vesicles under conditions resulting in a disturbance of the normal bilayer architecture [36, 37]. The biological significance of the binding specificity of CRP for PCh is underlined by the wide distribution of this group in eukaryotic cell membranes and procaryotic cell walls.

Available information on the structure of the PCh-binding site is indirect and inconclusive. On the basis of quantitative precipitation tests using PCh- and phosphoryl-ethanolamine-substituted bovine serum albumin and hapten inhibition assays [38], it was proposed that the binding site in CRP consists of a primary locus for a Ca^{++}-dependent binding of the phosphoryl group and a secondary locus for the binding of the cationic choline group. The zwitterionic nature of the PCh-binding site was subsequently confirmed by using phosphate monoesters, choline derivatives, and dipeptides as inhibitors

[39]. Ca^{++} is apparently acting as allosteric effector for the phosphate-binding site as indicated by circular dichroism [30] and immunochemical data [31]. Electron spin resonance studies indicated a shallow PCh-binding site which does not exceed 5 Å in depth and immunoelectron microscopy indicated that all of the PCh-binding sites are on the same face of the molecule and are nearly perpendicular to the plane of the molecule [40].

Additional binding specificities have been described for CRP. Of particular interest is the PCh-inhibitable binding to fibronectin [41] and to chromatin [42]. Both of these specificities have potentially important biological roles. A CRP molecule has been reported to bind 9 fibronectin molecules with a Kd of 1.47×10^{-7} *M* via the PCh binding site [43]. Binding of CRP inhibits attachment of normal rat kidney fibroblasts to fibronectin [44]. CRP pentamers were found to bind to chromatin with a Kd of 8×10^{-7} *M*, and with a stoichiometry of one CRP subunit per 160 base pairs of DNA [42]. It was shown later that CRP binds to histones H1, H2A, and H2B in a calcium-dependent manner [45] and to the 70-kDa polypeptide of the U1 small nuclear ribonucleoprotein [46]. It has been proposed that CRP reacts with these components in damaged tissues and aids in their removal through interactions with the complement system and cells of the phagocytic system.

Several laboratories have reported the binding of CRP to galactose-containing polysaccharides. These include de-pyruvylated pneumococcal type IV capsular polysaccharide [47], snail galactans [48], agarose [37], and *Leishmania* galactans [49]. The interaction of CRP with galactose derivatives is calcium-dependent and can be inhibited by PCh. Soelter and Uhlenbruck [50] reported that CRP binds to trace phosphate groups that are minor constituents of *Helix pomatia* galactan. It is not clear whether binding to other galactans is also directed to phosphate residues or alternatively, to carbohydrate determinants.

Binding of CRP to a variety of cationic substances has also been described [51]. The interaction is not calcium-dependent, and interestingly, the precipitation reaction between CRP and poly-l-lysine is enhanced by PCh [52].

Complement Activation by CRP Complexes

Activation of the complement system by CRP was first demonstrated for complexes of the protein with PnC and with phospholipids [34, 53]. Analysis of complement component depletion indicated that complement activation proceeded through the classical pathway. It was subsequently shown that complexes of CRP with a variety of other ligands including polycations [54], positively charged liposomes [55], PC:LPC liposomes [25, 37], and nuclear DNA [56] could also activate the classical pathway. We have also reported [57] that, similarly to immune aggregates, insoluble CRP-PnC precipitates can be solubilized by complement and that fragments of C3 bind covalently to CRP and to PnC during the solubilization reaction. In addition, formation of covalent complexes between CRP and the α' chain of C4 was also demonstrated [58]. Thus, CRP-initiated activation of the classical pathway of complement leads to the assembly of an effective C3-convertase and it seems reasonable to assume that it results in the generation of

host defense-related complement fragments, including the anaphylatoxins C3a and C4a and the opsonins C4b, C3b, and iC3b. However, CRP complexes do not appear able to lead to the formation of an efficient C5-convertase [59]. Thus, CRP-initiated complement activation may not generate the complement chemotactic factor C5a and the cell membrane lytic complex C5b-9.

Opsonic Properties of CRP

Initial experimental evidence indicating that CRP is an opsonin was provided by Lōfström [60, 61], who demonstrated that CRP induces the agglutination and capsular swelling of certain types of *Streptococcus pneumoniae* and 'gives a slight temporary protection to mice against infection with pneumococci types 27 and 28' [61]. Further studies indicated that certain bacterial species were ingested more efficiently by polymorphonuclear leukocytes after incubation with CRP [62]. These observations were expanded by Ganrot and Kindmark [63] and Kindmark [64], who used purified CRP and washed unfractionated blood leukocytes as a source of polymorphonuclear leukocytes. These investigators were able to clearly demonstrate that CRP enhances the phagocytosis of a variety of gram-positive and gram-negative pathogens. Mortensen et al. [65] reported that when both CRP and complement fragments were localized on the membranes of sheep red blood cells these cells were ingested by human monocytes. CRP alone had very little effect, and red cells from which the CRP, but not complement fragments, was removed by treatment with EDTA were found attached to the monocytes but were not ingested. Thus, CRP was thought to provide an additional signal necessary for phagocytosis to proceed. Edwards et al. [66] examined the opsonization of *S. pneumoniae* by CRP and subsequent interactions with isolated neutrophils in a chemiluminescence assay and concluded that the opsonic properties of CRP depended on its ability to activate complement. We have demonstrated that phagocytosis of CRP-opsonized sheep red blood cells by neutrophils requires prior stimulation of the neutrophils by either phorbol esters or a less than 10-kDa product(s) of stimulated blood mononuclear cells [26, 67]. This interaction is not mediated directly by the FcγIII receptor of the neutrophils and is not dependent on the presence of complement. We have also examined the ability of solid phase complexes of CRP and pneumococcal C-polysaccharide to stimulate degranulation of neutrophils and found a significant increase in degranulation of neutrophils bound to PnC-CRP as compared to neutrophils bound to PnC alone. Degranulation could be potentiated by treatment of the neutrophils with the low molecular weight factor(s) from stimulated mononuclear cells [68].

The relevance of the above-described studies on the in vitro opsonic properties of CRP to the in vivo function of the protein was demonstrated by using a murine pneumococcal infection model. Human CRP was shown to protect mice against fatal infection with type 3 and type 4 *S. pneumoniae* [69, 70]. Additional studies indicated that not only human but also rabbit CRP could mediate the blood clearance of pneumococci in mice [71]. Optimal activity required a functioning complement system and could be demonstrated in *xid* mice, which have virtually no naturally occurring antipneumococcal antibodies [71, 72].

Cellular Receptors for CRP

Initial studies on the opsonic properties of CRP were interpreted to indicate that it interacted with the Fc receptors of mouse monocytes [73]. More recently, Muller and Fehr [74] also reported interaction of CRP with the Fc receptors of human neutrophils. We reported [26] that neither 3G8, a monoclonal antibody against the FcγIII receptor, nor monomeric IgG_1 inhibited the binding of CRP-opsonized cells to neutrophils, while immune complexes containing rabbit IgG did inhibit CRP binding. However, CRP had no effect on the binding of IgG-opsonized cells to neutrophils [26]. We interpreted these data to indicate that there was a unidirectional interaction of putative CRP receptors and Fc receptors. More recent studies have described similar types of interactions between CRP and U937, a monocytic cell line [75], human monocytes [76], and neutrophils [77, 78]. In addition, several laboratories have examined the direct binding of radiolabeled CRP to neutrophils [74, 77, 78] and monocytes [75, 79, 80]. However, the exact mechanisms involved and specifically the nature of the putative receptor have not been determined. The critical interpretation of the data from these more recent binding studies is difficult due to differences in the assay systems utilized by different laboratories, but it is clear that CRP binds in a reversible manner.

There are limited data on the structure of the putative CRP receptor of U937 [75]. Tebo and Mortensen [75] described the cross-linking of CRP to a 40-kDa membrane protein and isolation of two membrane proteins, of 40 and 58–60 kDa by CRP-affinity chromatography. Interestingly, while IgG_1 inhibited the cross-linking of CRP to the 40-kDa protein, IV.3, a monoclonal antibody which inhibits binding of immune complexes to FcγII receptor, did not. In addition, proteins purified by CRP-affinity chromatography were distinguishable from isolated FcγI receptor.

Overall, these data indicate that CRP binds to phagocytic cells in a specific and reversible manner and that upon binding a biological response in the form of a phagocytic signal is elicited. Thus, they provide support for the presence of a specific receptor for CRP on phagocytic cells. The relationship of the receptor for CRP with Fc receptors is not clear presently.

CRP and Platelet-Activating Factor

In addition to its role as an opsonin, CRP has been postulated to play a protective role in the early stages of an inflammatory reaction [81–84] by inhibiting platelet-activating factor (PAF), 1-O-alkyl-2-O acetyl-*sn*-glycero-3-phosphocholine. Hokama et al. [81] first reported the inhibition of PAF-stimulated platelet aggregation by CRP. Subsequently, Vigo [82] reported that CRP also inhibited release of arachidonic acid from both phosphatidylcholine and phosphatidyl inositol of PAF-stimulated platelet membranes. Filep and Foldes-Filep [84] and Tasumi et al. [85] reported that CRP inhibited PAF-induced degranulation and superoxide anion production by human neutrophils. The effect of CRP was attributed to inhibition of the binding of PAF to neutrophils [83]. It was also shown in these studies that CRP protected neutrophils and platelets from the lytic effects of lysolecithin [82, 84]. The in vivo significance of the CRP-PAF interactions has not been determined, but the hy-

pothesis that, in addition to its role in host defense and recognition and elimination of damaged cells, CRP also plays a role in control of the inflammatory response is supported by these in vitro data.

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Medical Encyclopedia: Stroke secondary to atherosclerosis

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Definition

Loss of neurologic functions (brain attack) which occurs suddenly or in a step-wise fashion, caused by complications of atherosclerosis.

Causes, incidence, and risk factors

Stroke secondary to atherosclerosis affects about 2 out of 1,000 people, or approximately 50% of all those who have strokes. Strokes are the third leading cause of death in most developed countries, including the U.S. Stroke secondary to atherosclerosis is most common in people over 50 years old. The incidence of stroke rises dramatically with age, with the risk doubling with each decade after 35 years old. About 5% of people over 65 years old have had at least one stroke. The disorder occurs in men more often than women.

Atherosclerosis (hardening of the arteries) is a condition where fatty deposits occur in the inner lining of the arteries, and atherosclerotic plaque (a mass consisting of fatty deposits and blood platelets) develops. The plaque may obstruct (occlude) the artery by itself, or may trigger a clot (thrombus) at that location, causing cerebral thrombosis (thrombotic stroke). The occlusion of the artery develops slowly.

Atherosclerotic plaque does not necessarily cause stroke. There are many small connections among the various brain arteries. If blood flow gradually decreases, these small connections will increase in size and "by-pass" the obstructed area (collateral circulation). If there is enough collateral circulation, even a totally blocked artery may not cause neurologic deficits. A second safety mechanism within the brain is that the arteries are large enough that 75% of the blood vessel can be occluded, and there will still be adequate blood flow to that area of the brain.

Atherosclerosis occludes the blood vessels, causing ischemia (reduced tissue oxygenation associated with insufficient blood flow) and infarction (tissue death associated with ischemia).

Pieces of atherosclerotic plaque or clot may travel in the bloodstream (embolism). However, strokes caused by embolism are most commonly strokes secondary to cardiogenic embolism (clots that develop because of heart disorders, which then travel to the brain). Whatever the source of the embolism, the clot travels through the bloodstream and becomes stuck in a small artery in the brain. This stroke occurs suddenly with immediate maximum neurologic deficit (loss of brain function).

Risks for stroke secondary to atherosclerosis include: a history of high blood pressure (hypertension is present in about 70% of all victims of stroke); peripheral vascular disease; smoking; transient ischemic attacks or other cerebrovascular disease; high blood lipids; high levels of homocysteine; diabetes mellitus; obesity; sedentarism, and kidney disease requiring dialysis.

Symptoms

- weakness or total inability to move a body part
- numbness, loss of sensation
- tingling or other abnormal sensations
- decreased or loss of vision, which may be partial and/or temporary
- language difficulties (aphasia)
- inability to recognize or identify sen ory stimuli (agnosia)
- loss of memory

- facial paralysis
- eyelid drooping
- vertigo (abnormal sensation of movement)
- loss of coordination
- swallowing difficulties
- personality changes
- mood and emotion changes
- urinary incontinence (lack of control over bladder)
- lack of control over the bowels
- consciousness changes:
    - sleepy
    - stuporous, somnolent, lethargic
    - comatose, unconscious

Signs and tests

Signs of stroke are present. Testing is the same as for stroke. Serum lipids, especially triglycerides and cholesterol, may be high.

Other tests and procedures:

- head CT scan
- head MRI
- ECG (electrocardiogram) may be used to determine underlying heart disorders
- echocardiogram (if the cause is suspected to be cardiac embolus)
- carotid duplex (ultrasound)
- transcranial doppler (helps determine the caliber of the vessels inside the brain)

Treatment

Go to the emergency room as quickly as possible if you believe you have had or may be having a stroke. Stroke is an acute, serious condition that should be treated immediately. Strokes are now called "brain attacks" to stress that time is of the essence in treating this condition.

The most effective treatment for stroke (intravenous rtPA), which works to dissolve the offending clot and prevent permanent deficits, can only be given before three hours have elapsed since the onset of the deficits. There is risk of serious bleeding with this treatment so it cannot be used in all cases, but the most important factor in effective treatment for stroke is arriving at the hospital as early as possible from the onset of symptoms. For virtually all strokes, there is a need for hospitalization, possibly including intensive care and life support.

For patients who can't be treated with clot-busting drugs, treatment will be based on the type of stroke they may have had, however, the focus will be supportive (i.e., blood pressure control, adequate fluid management, and prevention of complications such as infections). Rehabilitation is important following stroke to maximize function in affected areas. Treatment is also aimed at prevention of future strokes. Recovery may occur as other areas of the brain take over functioning for the damaged areas. The goal of treatment is to prevent spread (extension) of the stroke and to maximize the ability of the person to function (see stroke).

Special treatment (in addition to treatment for stroke in general) may include medications to control blood cholesterol levels.

A special diet often follows the American Heart Association recommendations for people with hyperlipidemia (increased fats/lipids in the bloodstream). This may include restriction of fat, especially saturated fat. It may also include restriction of salt/sodium if stroke is accompanied by high blood pressure.

A carotid endarterectomy (removal of plaque from the carotid arteries) may be needed by some people to prevent new strokes from occurring.

Expectations (prognosis)

Stroke is the third leading cause of death in developed countries. About one-fourth of the sufferers die as a result of the stroke or its complications, about one-half have some degree of recovery but are left with variable disability and about one-fourth recover most or all function. There are three important signs occurring at the onset of the stroke that predict best who will recover to a lesser extent in the convalescence phase: low-grade temperature or fever, high blood sugar (hyperglycemia), and low blood pressure (hypotension). People with these symptoms are less likely to make a full recovery. Contrary to common belief, recurrence of stroke is only around 2% within the first 14 days.

Complications

- pressure sores
- permanent loss of movement or sensation of a part of the body
- orthopedic complications, fractures, contractures, muscle spasticity
- permanent loss of cognitive functions
- disruption of communication, decreased social interaction
- decreased ability to function or care for self
- decreased life span
- multi-infarct dementia
- side effects of medications

Calling your health care provider

Go to the emergency room or call the local emergency number (such as 911) if symptoms occur indicating a stroke.

Prevention

The prevention of stroke secondary to atherosclerosis includes control of risk factors. Hypertension, diabetes, heart disease, and other risk factors should be treated as appropriate. Smoking should be minimized or, preferably, stopped.

Treatment of TIA can prevent some strokes.

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